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RESEARCH ARTICLE

Trace metal contamination in a Mediterranean coastal pond (Acquatina, Puglia)

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Abstract

1 - As the interface between land and sea, transitional areas are often negatively impacted by anthropic activities. This impact is the result of many processes, including direct input of pollutants which accumulate in both sediments and aquatic organisms.

2 - The aim of this paper is to identify trace metal (As, Cd, total Hg and Pb) contamination in the Acquatina pond (Apulia, Italy), using sediment and fish (*A. boyeri, C. labrosus, D. annularis, D. dentex,, D. sargus, D. vulgaris, E. encrasicholus, G. paganellus, L. ramada, M. surmuletus, S. salpa, S. solea*) as descriptors. Sampling was carried out in three stations (South, Central and North) and four seasons (summer and autumn 2007, winter and spring 2008). Fish metal concentrations were correlated with $\delta^{15}N$ values, which is an indicator of their trophic position.

3 - Both sediment and fish trace metal concentrations were compared with regulatory limits. Results showed that trace metal concentrations in sediment were comparable to levels found in moderately contaminated areas. The North station was more contaminated, probably because of freshwater discharges through the Giammatteo channel.

4 - Within ichthyofauna, higher metal levels were found in demersal fish. A significant relationship between trace metal and $\delta^{15}N$ was found only in total Hg in demersal fish.

Keywords: trace metals, pollution, sediment, fish, stable nitrogen isotopes, transitional waters

Introduction

Coastal ponds, along with other complex ecosystems (lagoons, estuaries, bays etc.), are defined as confined areas characterized by shallow depth and widely variable environmental features (e.g. temperature, salinity, dissolved oxygen). Common characteristics are also high rates of primary and secondary productivity, environmental complexity and high resilience (Nybakken, 1993).

Despite their ecological importance, coastal ponds are typically vulnerable ecosystems, often suffering from considerable anthropogenic pressure. Due to the potential input of fertilizers, pesticides and fungicides and other chemicals containing trace metals, assessment of chemical contamination is particularly significant in these areas. Sediments can act as important sinks for trace metals, making analytical determination of their concentrations straightforward and supplying time-integrated information about ecosystem health (Burton, 1992). Trace metals also tend to be accumulated within the biota. In particular, their absorption by fish is influenced by biotic factors such as species (Türkmen *et al.*, 2005), habitat (Romeo *et al.*, 1999), diet (Kirby *et al.*, 2001), life cycle stage (Farkas *et al.*, 2002, 2003) and sex (Al-Yousuf *et al.*, 2000), but also by physic-chemical factors (Rubio *et al.*, 1996; Rauret, 1998) and by the chemical form of metals (Ahlf and Forstner, 2001).

Levels of contaminants in fish are of considerable interest because of potentially negative effects on the fish themselves and on higher trophic level predators (e.g. birds, humans). The accumulation of metals is often observed to increase up through the food web, a phenomenon attributed to biomagnification. In this context, the coupling of isotopic ($\delta^{15}N$) and contaminant determinations provides a tool to assess the trophic transfer of pollutants within food webs and to analyse bioaccumulation and biomagnification processes (e.g. Tittlemier *et al.*, 2002). In fact, $\delta^{15}N$ can fairly accurately indicate the trophic level of species within an ecosystem (Kling *et al.*, 1992; Cabana and Rasmussen, 1994).

This paper reports the concentrations of four metals (As, Cd, total Hg and Pb) in surface sediments and in twelve fish species from a Mediterranean coastal pond (Acquatina, Apulia Italy). Metal levels in fish were correlated with their $\delta^{15}N$ in order to assess their variation with trophic level.

Materials and methods

Study site and sample collection

Acquatina is a coastal pond located on the Adriatic side of the Salento Peninsula, close to the city of Lecce in Apulia (Italy). It has an extension of 45 hectares, and is connected to the adjacent marine area through two channels: a northern one (Giammatteo channel), from which it also receives freshwater, and a southern one. The bottom is muddy and the average depth is 1.2 m with a maximum of 2 m.

Sampling was carried out in three stations (Fig. 1): one close to the southern channel (South Station - S), another close to the northern channel (North Station - N) and the third in the central area (Central Station - C). At each station fish were collected seasonally (summer and autumn 2007, winter and spring 2008) using movable trap nets, the so-called bertovelli, while sediments were collected in June and November 2007 using hand corers.

Laboratory analyses

Fish were identified to species level and biometric variables measured. Fish were also classified according to their habitat into: demersal (*Atherina boyeri*, *Chelon labrosus*, *Diplodus sargus*, *Gobius paganellus*, *Mullus surmuletus*, *Solea solea*), benthopelagic (*Dentex dentex*, *Diplodus annularis*, *Diplodus vulgaris*, *Sarpa salpa*) and pelagic (*Liza ramada*, *Engraulis encrasicolus*). Only dorsal muscle was processed for analysis.

Before metal analysis, sediment and fish muscle were rinsed with distilled water, then oven dried to 40°C for 48 h. For isotope analysis, all samples were oven dried to 60°C and ground to a fine powder using a mortar and pestle. All analyses were conducted in triplicate.

Isotopic composition

Nitrogen stable isotope ratios ($\delta^{15}N$) were analysed using an Isotope Ratio Mass Spectrometer (IRMS; Thermo-Electron Delta Plus XP) coupled to an Elemental Analyser (EA; Thermo-Electron 1112). Results were expressed in δ notation according to this equation:

$^{x}\delta = \delta^{x}E = [(R_A - R_r)/R_r]\ (10^3)$

where: E = element, x = heavy isotope mass, R = ratio $^{15}N/^{14}N$ in the sample (A) and reference material.

Analytical precision was 0.2‰.

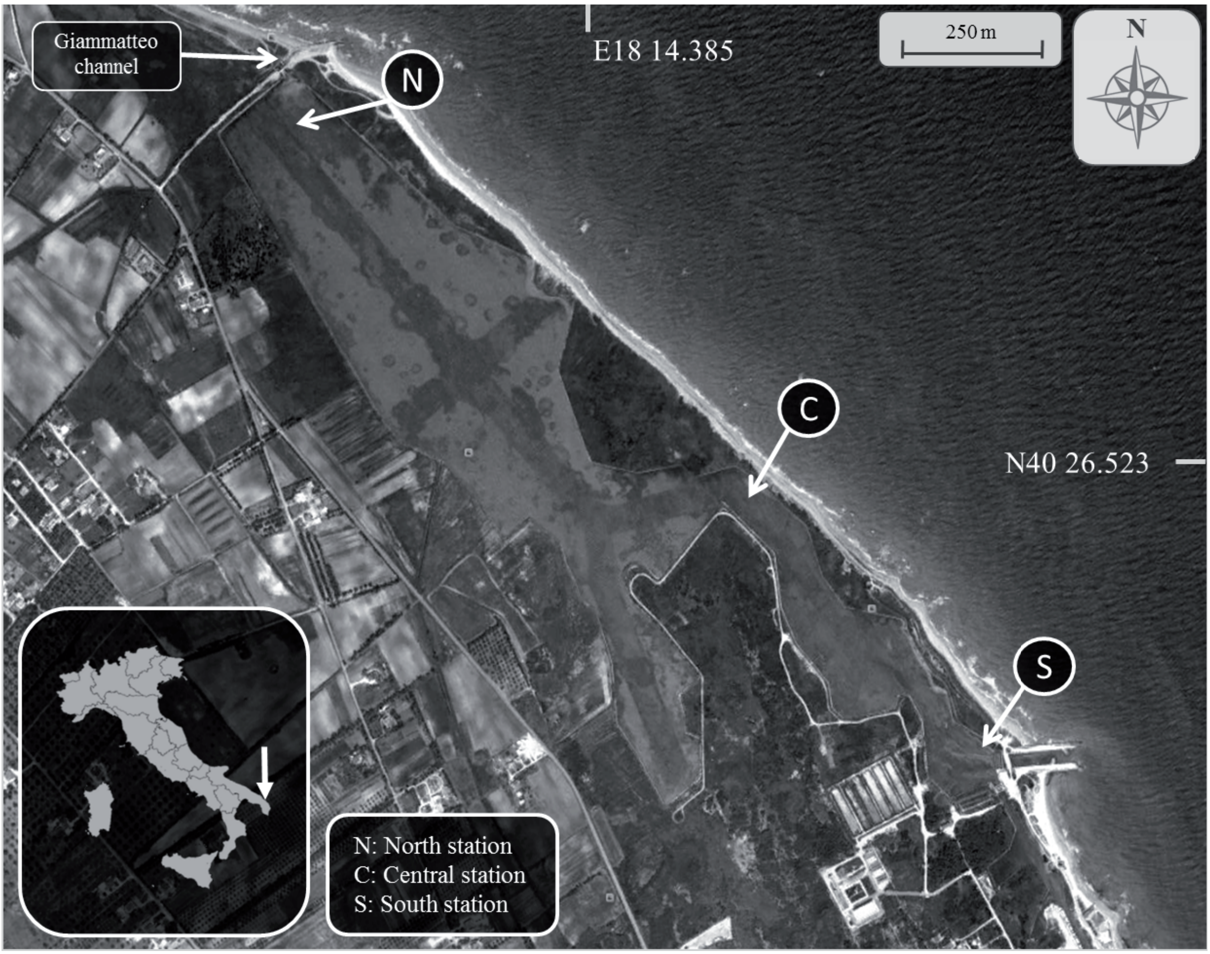

Figure 1. Acquatina coastal pond with sampling stations.

Trace metals

Trace metals were determined using a Varian Vista MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). Biological samples (about 0.5 g) were mineralized in Teflon digestion vessels with 5 mL of 67–70% HNO_3, 1 mL of 30% H_2O_2 and 4 mL of MilliQ deionized water, while sediments (about 0.2 g) were mineralized with 9 mL of 67–70% HNO_3, 3 mL of 40% HF, 0.5 mL of H_2O_2 30% and 2.5 mL of MilliQ deionized water. After cooling, each sample was transferred to a volumetric flask (25 mL for biological samples and 50 mL for sediments) and diluted with distilled water. For each cycle of mineralization an analytical blank was prepared. The analytical procedure was checked using a standard reference material: Dogfish muscle DORM-2 (provided by the National Research Council of Canada) for fish and Marine Sediment MESS-3 (National Research Council of Canada) for sediment. Concentrations of As and total Hg were determined using a hydride generation system (VGA-77 linked ICP-OES). For As determinations, 4 mL of 5% KI, 4 mL of 5% ascorbic acid and 4 mL of 5% HCl were added in that sequence to 4 mL of the digested sample to convert total As to As^{3+}. After that, As^{3+} was transformed to AsH_3 by adding 0.6% $NaBH_4$ and 0.5% NaOH as reductants and 5–10 M HCl as carrier. For total Hg determinations, a reductant solution, consisting of 0.3% NaBH4 and 0.5% NaOH, and 5 M HCl as carrier, was used. All reagents were Suprapur. Results were given in mg kg^{-1} d.w.

Statistical analysis

Cluster analysis was carried out on trace metal data in sediment, after normalization and the creation of a triangular matrix based on Euclidean distances.

Linear regressions were carried out to investigate the correlation between trace metals (based on logn) and $\delta^{15}N$ of fish species subdivided according to their habitat (demersal, benthopelagic, pelagic).

Data of trace metals in fish were analysed in a multivariate context. Classification factors used were: species, station, season and habitat. These were first normalized and then a triangular matrix obtained using Euclidean distances. Data were subjected to nMDS (non-metric multi dimensional scaling) using the software PRIMER 6 (Plymouth Routines in Marine Ecological Research) (v. 5.2) (Clarke and Gorley, 2006).

Results

Metal levels in sediment are shown in Fig. 2. Total concentrations were higher in the North station, while they were similar in the South and Central stations. Differences between the North and the other stations were much higher for As (about 8-fold higher) than for other elements (about 2). This general trend was confirmed by cluster analysis, which showed that the South station was more similar to the Central than to the North one (Fig. 3).

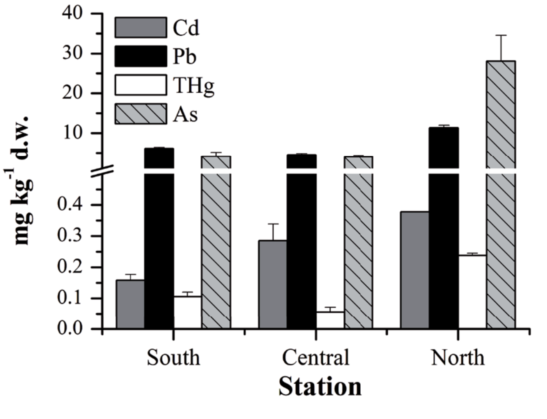

Figure 2. Mean (± sd) Cd, Pb, total Hg and As levels (mg kg^{-1} d.w.) in sediments from Acquatina.

Seasonally averaged trace metal levels in fish muscle are given in Fig. 4, while all data subdivided by station and season are presented in the Appendix. In general, a higher accumulation of Cd was detected in samples from the North station, while a spatial pattern was undetectable for the other metals. Maximum total Hg concentrations were found in *Atherina boyeri* (1.84±0.63 mg kg^{-1} d.w.), while the species that showed the lowest levels for all metals was Solea solea. nMDS did not show a clear effect for several factors (i.e. species, season, station and habitat) on contamination level of the ichthyofauna (Fig. 5).

Linear regressions between trace metal concentrations recalculated on logn and $\delta^{15}N$ values in fish subdivided into demersal, benthopelagic and pelagic are reported in Figs. 6-8. A significant positive correlation ($R=0.61$, $p<0.05$) was found only in total Hg in demersal fishes (Fig. 6).

Discussion

Trace metal levels in sediments classify Acquatina as a moderately impacted site. A comparison of metal concentrations in sediments from the study area with literature data and regulatory limits is shown in Table 1. As and Cd concentrations are respectively higher than and similar to those reported in sediments from the Berre Lagoon (Accornero *et al.*, 2008), which is considered a contaminated area by the French Réseau National d'Observation de la qualité du milieu marin (National network for the observation of marine environmental quality). Cd, Pb and total Hg levels in Acquatina sediments are lower than those found in the contaminated lagoon Ghar El Melh (Chouba *et al.*, 2007). Pb levels are lower than most Greek lagoons examined by Christophoridis *et al.* (2007). In addition, all metals had levels far below those found in Venice Lagoon and in its highly contaminated industrial canal (Bellucci *et al.*, 2002).

Trace metal contamination in sediment are slightly higher than the Italian regulatory limits (G.U.R.I., D.M. 367/03, 2004). As and Cd exceeded the legal limits (12 mg kg^{-1} d.w. and 0.3 mg kg^{-1} d.w., respectively) only in the North station; while total Hg and Pb did not exceed the limits (0.3 and 30 mg kg^{-1} d.w. respectively) in any station. With the aim of defining a potential level of biological risk associated with trace metal concentrations, we compared our results to the effects range low (ER-L) and effects range medium (ER-M) benchmarks (Tab. 1), according to marine sediment quality guideline values from NOAA's National Status and Trends Program (Long *et al.*, 1995). Averaged As and total Hg concentrations were higher than the ER-L values (8.2 and 0.15 mg kg^{-1} d.w. respectively) in the North station, while Cd and Pb did not exceed the benchmarks. Concentrations equal to, or greater than ER-L, but lower than ER-M, as in this study, represent a range within which biological effects (metals are known to cause toxic effects on a variety of tissues, such as nervous system) occasionally occur. Therefore NOAA guidelines suggest a

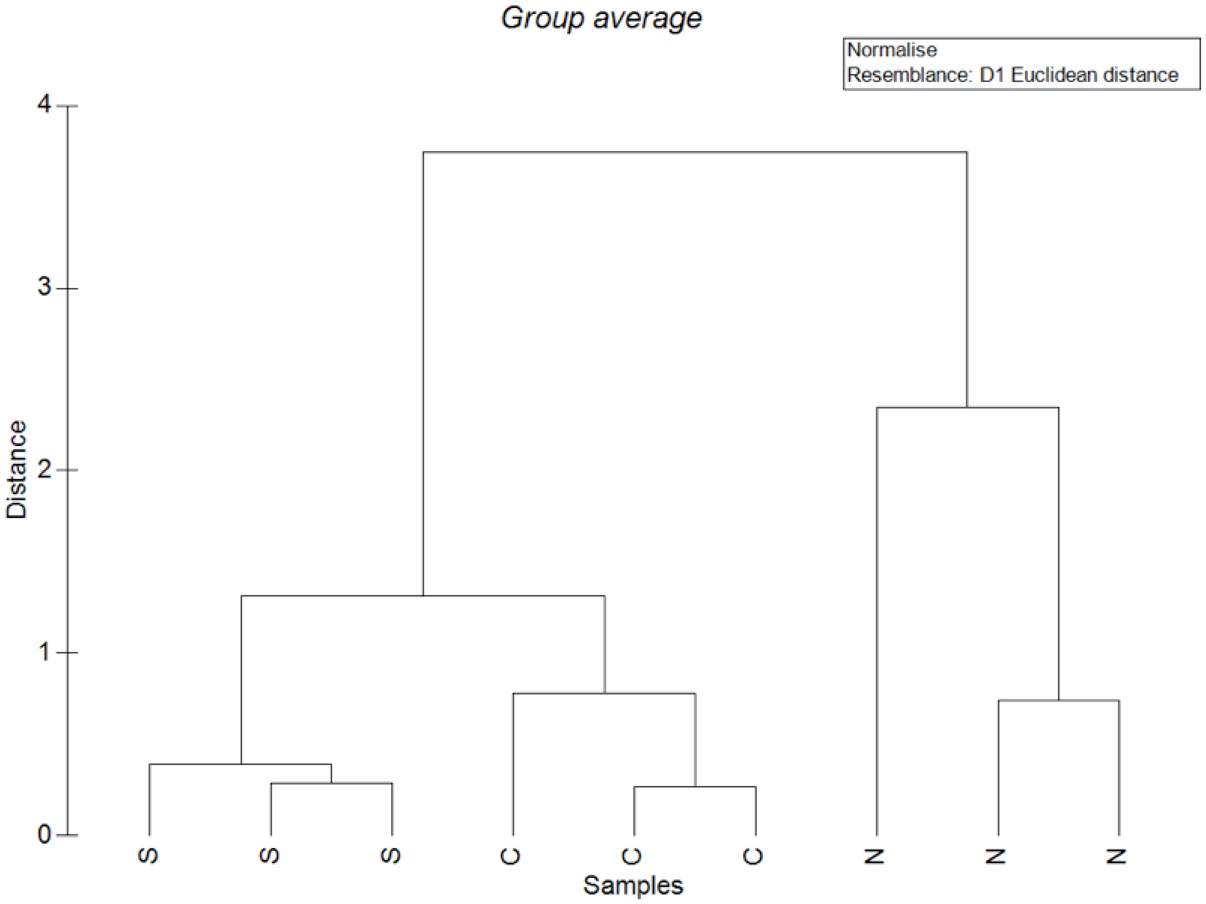

Figure 3.Cluster analysis based on normalized Euclidean distances of trace metal levels (Cd, Pb, total Hg and As) in sediments from Acquatina (S: South station, C: Central station, N: North station).

potential risk associated with total Hg and As, but not with Cd.

Spatial differences occurred in metal concentration within Acquatina. North station was more contaminated than Central or South. This result may account for the Giammatteo channel causing freshwater, and probably contaminant, input due to runoff from agricultural fields and anthropic discharges.

The direct transfer of chemicals from sediments to organisms is considered to be a major route of exposure for many species (Zoumis *et al.*, 2001). In this study we focused on trace metal levels in fish because of their importance for human consumption.

Table 1 - Ranges of trace metal levels (mg kg^{-1} d.w.) in sediments from Acquatina, in literature and regulatory limits.

	Cd	**Pb**	**THg**	**As**	**Reference**
Present study	0.14-0.57	4.28-11.97	0.04-0.25	3.11-34.13	
Lesina (Italy)	0.44-1.34	0.63-28.34	-	-	D'Adamo et al. (2008)
Agiasma	-	6.24-158.20	-	-	Christophoridis et al. (2007)
Venice	-	6.1-278	-	-	
Thau	-	13.8	-	-	
Gialova	-	0.04-1.13	-	-	
Rodia (Greece)	-	36	-	-	
Tsoukalio	-	26	-	-	
Logarou	-	25	-	-	
Tsopeli	-	26	-	-	
Messolonghi	-	12	-	-	
Kavala bay	-	30-670	-	-	
Berre (France)	0.2-1.6	18-82	0.15-0.40	4-10	Accornero et al. (2008)
Venice Lagoon (Italy)	0.2-5	38-114	1.2-1.4	5-25	Bellucci et al. (2002)
Canal Industrial area	0.3-70	21-929	2-48	5-132	
Ghar El Melh (Tunisia)	0.4-0.9	25-65	0.3-1.4	-	Chouba et al. (2007)
ER-L	1.2	47	0.15	8.2	NOAA (Long et al., 1995)
ER-M	9.6	220	0.71	70	
Italian Regulatory limits	0.3	30	0.3	12	n. 367/2003

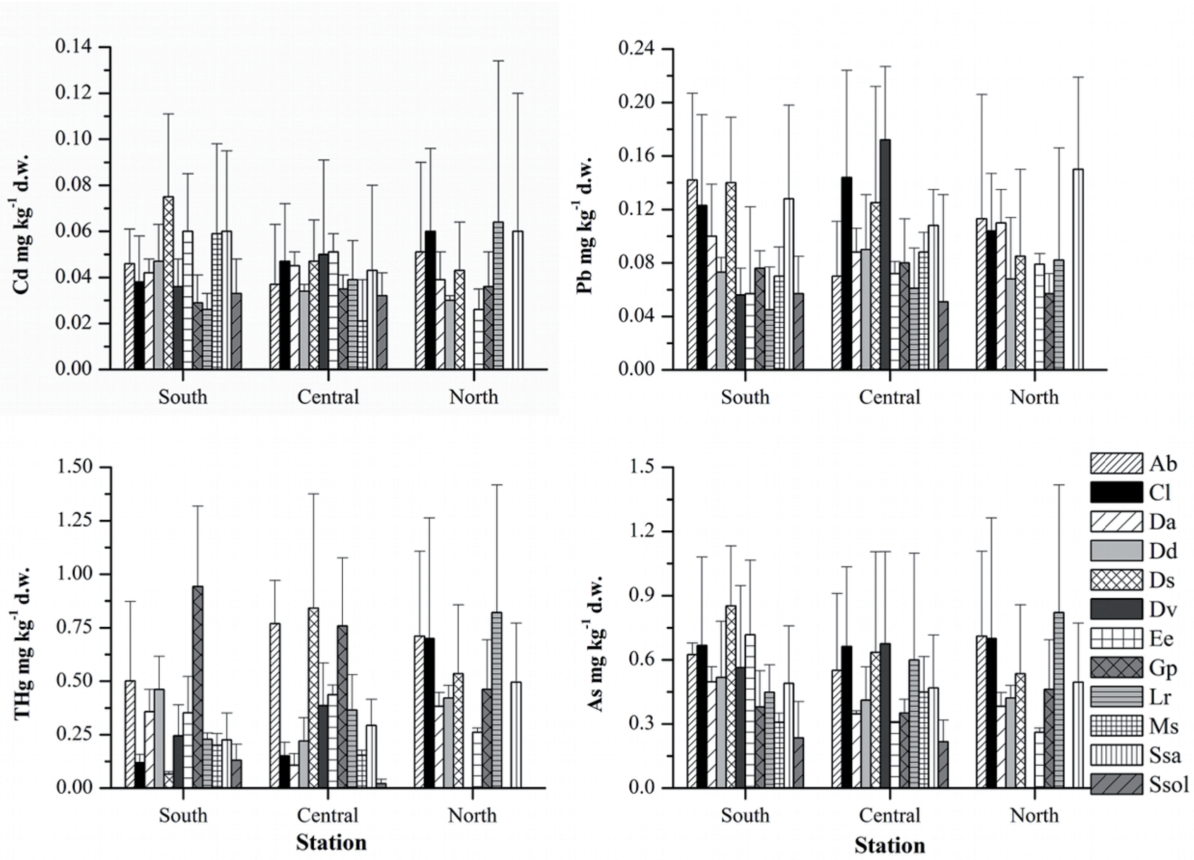

Figure 4. Mean (±sd) Cd, Pb, total Hg and As levels (mg kg^{-1} d.w.) in fish from Acquatina. Ab=*Atherina boyeri*; Cl=*Chelon labrosus*; Da=*Diplodus annularis*; Dd= *Dentex dentex*; Ds=*Diplodus sargus*; Dv=*Diplodus vulgaris*; Ee=*Engraulis encrasicolus*; Gp= *Gobius paganellus*; Lr=*Liza ramada*; Ms= *Mullus surmuletus*; Ssa=*Sarpa salpa*; Ssol= *Solea solea*.

Overall we found high variability in fish metal content between- and within-species. However, this variability was not explained by each single factor considered in the present study, such as species, station, season and habitat. Accordingly, in the literature, trace metal variability in fish is not recognized to be induced by a single factor, but by a combination of the physical and chemical status of sea water (Jazierska and Witeska, 2001), season (Çoğun *et al.*, 2006; Dural *et al.*, 2007; Dragun *et al.*, 2007), habitat(Romeo *et al.*, 1999; Kucuksezgin *et al.*, 2001) and detoxification ability (Dallinger *et al.*, 1987). In recent years, numerous studies have focused on the relationship between metal concentration in organisms and their trophic level (e.g. Atwell *et al.*, 1998; Das *et al.*, 2003; Taylor and Maher, 2003; Quinn *et al.*, 2003; Cabana and Rasmussen, 1996). In the present study, linear regressions between log-transformed trace metal concentrations and $\delta^{15}N$ as an estimate of trophic level emphasized non-significant correlations ($p>0.05$), apart from total Hg in demersal fish. This result suggests high total Hg levels in organisms living close to the bottom where Hg is recognized to be mainly present in its methylated form (i.e. the chemical species that undergoes biomagnification) (Gray, 2002; Bloom, 1992; Grieb *et al.*, 1990).

We compared trace metal levels in the 12 fish

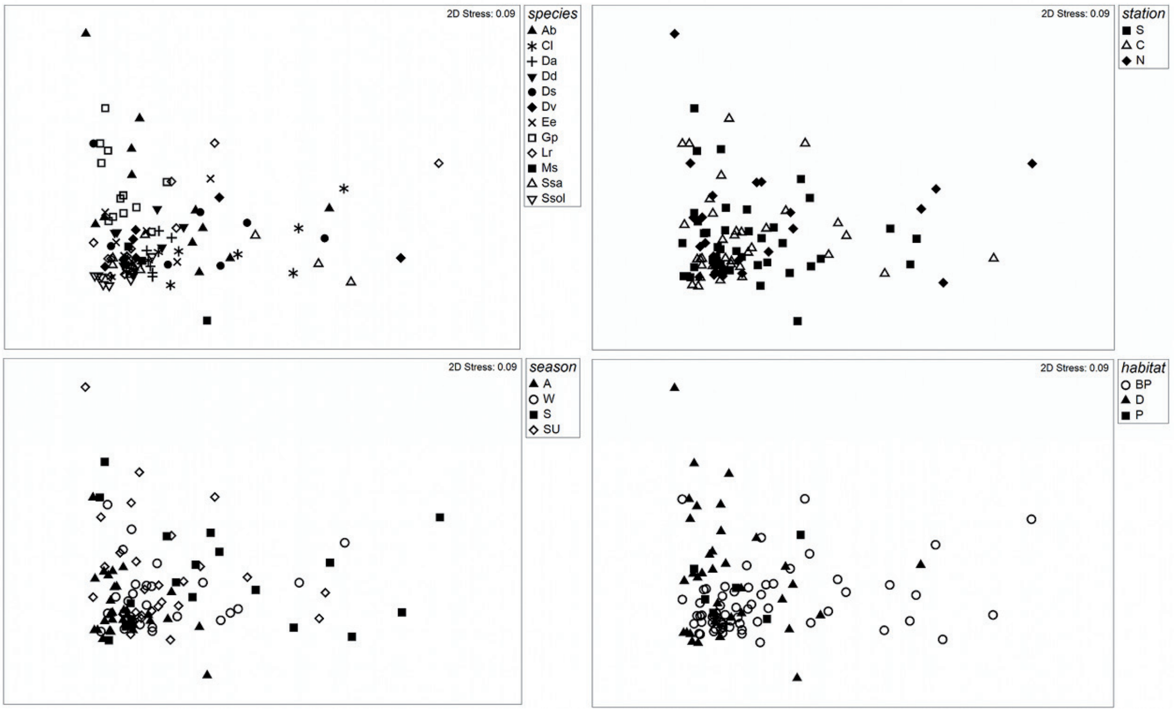

Figure 5. Non-metric multidimensional scaling ordination (nMDS) based on normalized Euclidean distances of trace metal levels (Cd, Pb, total Hg and As) in fish from Acquatina subdivided by species, station, season and habitat. For acronyms of species see Figure 4. S: South station, C: Central station, N: North station, A: autumn, W: winter, S: spring, SU: summer, BP: benthopelagic species, D: demersal species, P: pelagic species.

species analyzed following "EU Regulation n. 221/2002-amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs", which reports the maximum recommended levels of three trace metals (Cd, Hg and Pb) in the edible parts of fishery products. To compare the data of the present study (expressed as dry weight) with the limits (expressed as wet weight), we transformed the former according to the following equation, as suggested by Magalhães *et al.* (2007):

wet weight = dry weight * H_2O content in fish / 100

where H_2O content in fish is estimated as 78%. Using the above formula, we showed that in a few cases fish from Acquatina exceeded the limits permitted by law. In particular, averaged Cd content exceeded the maximum value of 0.05 mg kg^{-1} w.w. in 5 species (*Atherina boyeri*, *Diplodus sargus*, *Liza ramada*, *Mullus surmuletus* and *Sarpa salpa*), but never the value of 0.1 mg kg^{-1} w.w. Pb never exceeded the maximum concentration permitted, equal to 0.3 mg kg^{-1} w.w. Finally, Hg exceeded the maximum permitted (0.5 mg kg^{-1} w.w.) in 3 species (*Atherina boyeri*, *Diplodus sargus* and *Gobius paganellus*). As is not included in EU Regulation n.221/2002 so we compared its values with those in Bilandžˇić *et al.* (2011). After transformation to mg kg^{-1} w.w., As levels never exceeded the limit permitted for marine fish (2 mg kg^{-1} w.w.).

Herbicides, pesticides and fungicides are thought to be the main sources of As, according to the literature (Leist, 2000; Caussy, 2003; Castro-González and Méndez-

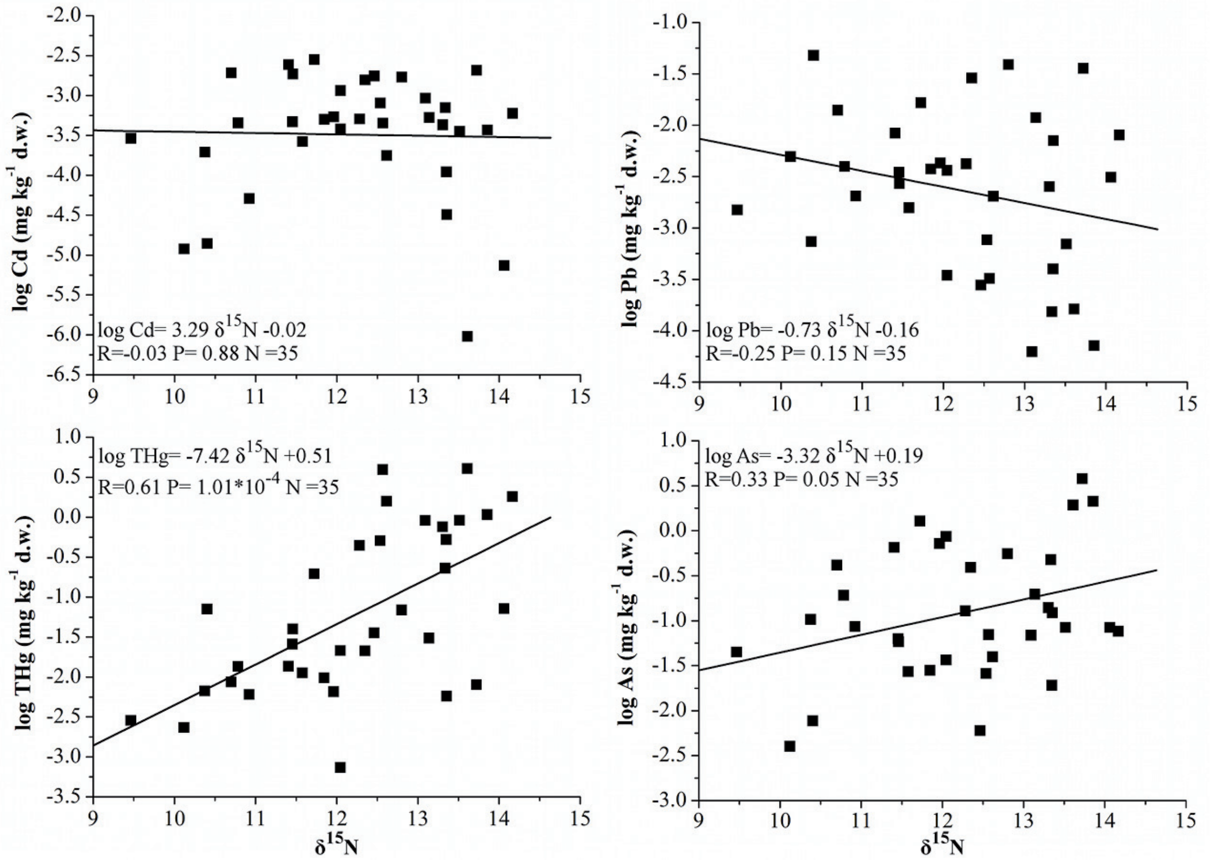

Figure 6. Linear regressions between logn-transformed Cd, Pb, total Hg and As levels and δ^{15}N in Acquatina demersal fish.

Armenta, 2008). However, although non-negligible As concentrations were found in sediments, as revealed by comparison with Italian Ministry Decree n. 367/2003 and NOAA Guidelines, its transfer to fish resulted low. In contrast, although Cd showed lower levels than those having biological effects, a few fish (5.3% of total) were found to be slightly contaminated, yielding values exceeding permitted levels. Higher Cd concentrations, especially in the North station, are affected by low salinity due to freshwater input. Indeed, Cd bioavailability increases in freshwater as a consequence of lower Cl concentration, as Cl forms complexes with Cd, reducing its bioavailability (Rainbow and Black, 2005). As reported by ATSDR (2003), the origin of Cd is by industrial emissions, fertilizer and sewage sludge. No environmental concerns were highlighted for Pb. Finally, Hg showed low concentrations in sediments, although these suggest a potential biological risk and 9.4% of total fish exceeded regulatory limits. In the study site the origin of Hg is probably due to agricultural activities.

In conclusion, considering the important role of Acquatina as a nursery and feeding area for many fish species (Maci and Basset, 2009), accurate monitoring and consequent management actions are fundamental in order to prevent further contamination and major biological effects. Particular attention is needed in order to prevent the quality of freshwater input from continental system, because, due to the wide anthropic pressure in coastal areas, river courses

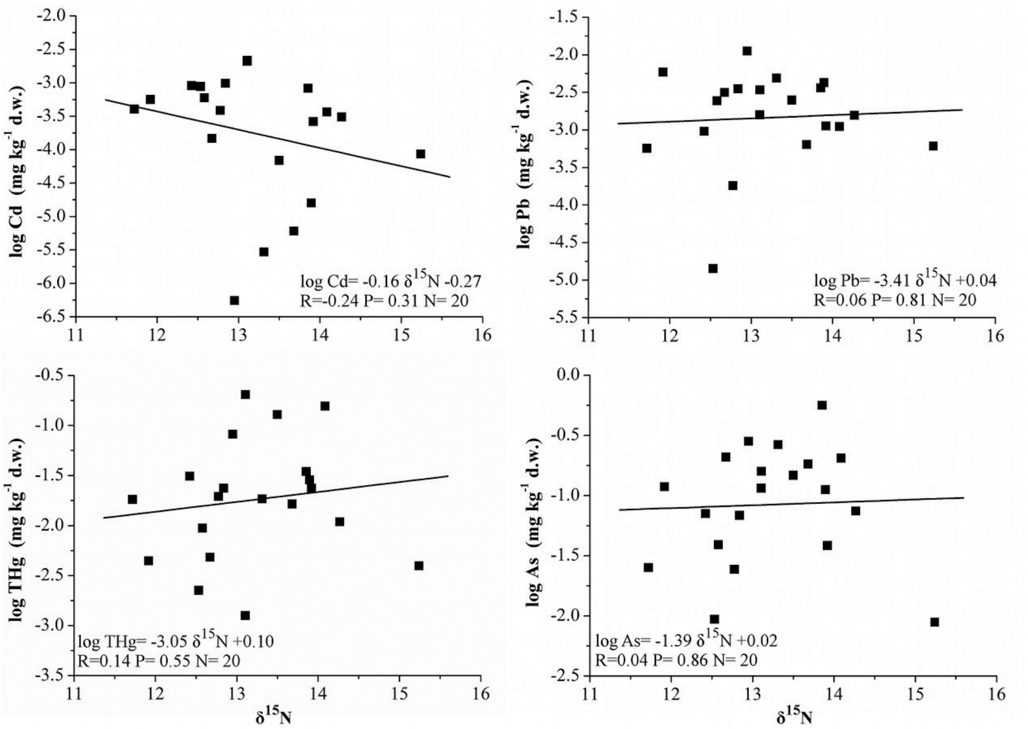

Figure 7. Linear regressions between logn-transformed Cd, Pb, total Hg and As levels and δ^{15}N in Acquatina benthopelagic fish.

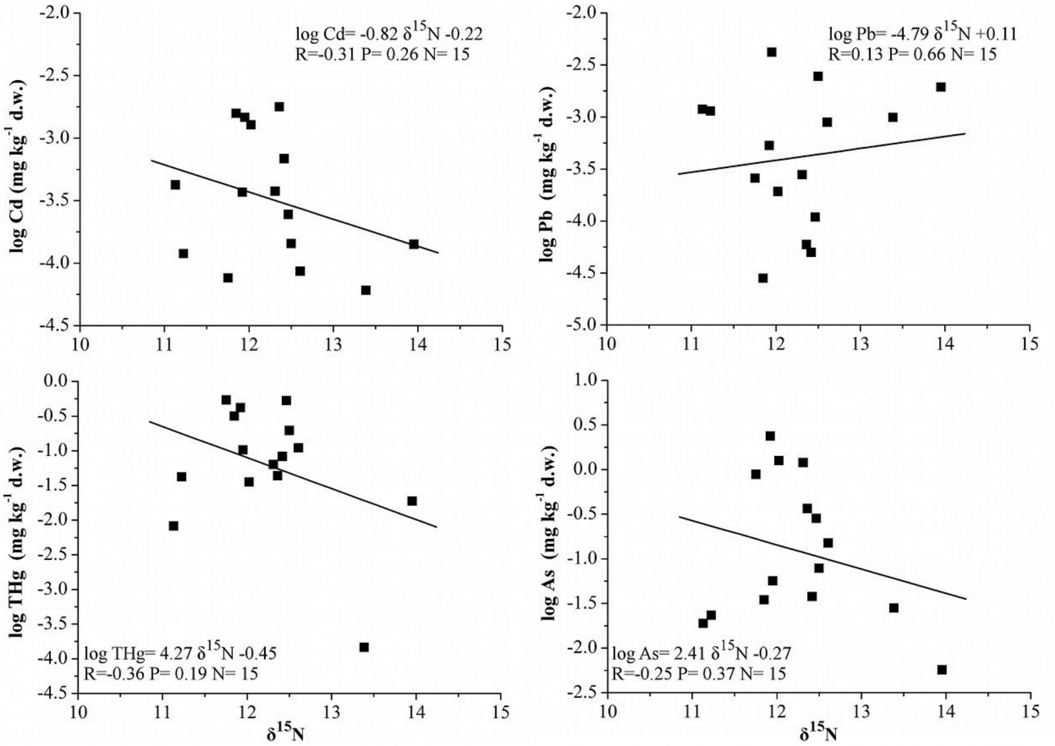

Figure 8. Linear regressions between logn-transformed Cd, Pb, total Hg and As levels and δ^{15}N in Acquatina pelagic fish.

and also small streams are repositories of large quantities of diverse contaminants.

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References

Accornero A, Gnerre R, Mandra L 2008. Sediment Concentrations of Trace Metals in the Berre Lagoon (France): An Assessment of Contamination. *Archives of Environmental Contamination and Toxicology* 54: 372–385.

ATSDR - Agency for Toxic Substance and Disease Registry, 2003. Toxicological Profile for Cadmium, U.S. Department of Health and Humans Services, Public Health Service, Centres for Diseases Control, Atlanta, GA.

Ahlf W, Forstner U 2001. Managing contaminated sediments. *Journal of Soils and Sediments* 1: 1–7.

Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM 2000. Trace metals in liver, skin and muscle of *Lethrinus lentjian* fish species in relation to body length and sex. *Science of the Total Environment* 256: 87-94.

Atwell L, Hobson KA, Welch HE 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1114-1121.

Bellucci LG, Frignani M, Paolucci D, Ravanelli M., 2002. Distribution of heavy metals in sediments of the Venice Lagoon: the role of the industrial area. *Science of the Total Environment* 295: 35-49.

Bilandžˇić N, Dokić M, Sedak M 2011. Metal content determination in four fish species from the Adriatic Sea. *Food Chemistry* 124: 1005–1010.

Bloom NS 1992. On the chemical from of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1131– 1140.

Burton GA Jr 1992. Sediment toxicity assessment. Lewis Publishers, Chelsea, MI.

Cabana G, Rasmussen JB 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372: 255-257.

Cabana G, Rasmussen JB 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences USA* 93: 10844-10847.

Castro-González MJ, Méndez-Armenta M 2008. Heavy metals: Implications associated to fish consumption. *Environmental Toxicology and Pharmacology* 26: 263–271.

Caussy D 2003. Case studies of the impact of understanding bioavailability: arsenic. *Ecotoxicology and Environmental Safety* 56: 164–173.

Chouba L, Kraiem M, Njimi W, Tissaoui CH, Thompson JR, Flower RJ 2007. Seasonal variation of heavy metals (Cd, Pb and Hg) in sediments and in mullet, *Mugil cephalus* (Mugilidae), from the Ghar El Melh Lagoon (Tunisia). *Transitional Waters Bulletin* 4: 45-52.

Christophoridis A, Stamatis N, Orfanidis S 2007. Sediment heavy metals of a Mediterranean coastal lagoon: Agiasma, Nestos Delta, Eastern Macedonia (Greece). *Transitional Waters Bulletin* 4: 33-43.

Clarke KR, Gorley RN 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Çoğun HY, Yűzereroğlu TA, Firat Ő, Gők G, Kargin F 2006. Metal concentrations in fish species from the northeast Mediterranena Sea. *Environmental Monitoring and Assessment* 121: 431–438.

Dallinger R, Prosi F, Segner H, Back H 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia* 73: 91-98.

Das K, Beans C, Holsbeek L, Mauger G, Berrow SD, Rogan E, Bouquegneau JM 2003. Marine mammals from northeast atlantic: relationship between their trophic status as determined by $\delta^{13}C$ and $\delta^{15}N$ measurements and their trace metal concentrations. *Marine Environmental Research* 56: 349-365.

Dragun Z, Raspor B, Podrug M 2007. The influence of the season and the biotic factors on the cytosolic metal concentrations in the gills of the European chub (*Leuciscus cephalus* L.). *Chemosphere* 69: 911–919.

Dural M, Göksu MZL, Özak AA 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chemistry* 102: 415-421

EEC 2001. Commission Regulation (EC) n. 466/2001of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities.

EU Regulation n. 221, 6 February 2002. Official Journal of the European Communities.

Farkas A, Salanki J, Specziar A 2002. Relation between growth and the heavy metal concentration in organs of bream *Abramis brama* L. populating Lake Balaton. *Archives of Environmental Contamination and Toxicology* 43: 236-243.

Farkas A, Salanki J, Specziar A 2003. Age and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Research* 37: 959-964.

Gray JS 2002. Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin* 45: 46–52.

Grieb TM, Driscoll CT, Gloss SP, Schofield CL, Bowie GL, Porcella DB 1990. Factors affecting mercury accumulation in fish in the Upper Michigan Peninsula. *Environmental Toxicology and Chemistry* 9: 919–30.

G.U.R.I. - Gazzetta Ufficiale della Repubblica Italiana 2004. Decreto Ministeriale 6 novembre 2003, no 367. Regolamento concernente la fissazione di standard di qualità nell'ambiente acquatico per le sostanze pericolose, ai sensi dell'articolo 3, comma 4, del decreto legislativo 11 maggio 1999, n. 152, vol. 5. In Ministero dell'Ambiente e della Tutela del Territorio, Roma, pp. 17–29. 08 Gennaio.

Jerieska B, Witeska M 2001. Metal toxicity to fish. University of Podlasie. Monografie 42.

Kirby J, Maher W, Krikowa F 2001. Selenium, cadmium, copper, and zinc concentrations in sediments and mullet (*Mugil cephalus*) from the southern basin of lake Macquarie, NSW, Australia. *Australian Archives Enviromental Contamination and Toxicology* 40: 246-256.

Kling GW, Fry B, O'Brien WJ 1992. Stable isotopes and planktonic trophic structures in Arctic lakes. *Ecology* 73: 561-566.

Kucuksezgin F, Altay O, Uluturhan E, Kontas A 2001. Trace metal and organochlorine residue levels in red mullet (*Mullus barbatus*) from the Eastern Aegean, Turkey. *Water Research* 35: 2327–2332.

Leist M, Casey RJ, Caridi D 2000. The management of arsenic wastes: problems and prospects. Journal of Hazardous *Materials* B76: 125–138.

Long ER, MacDonald DD, Smith SL, Calder FD 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management* 19: 18–97.

Maci S, Basset A 2009. Composition, structural characteristics and temporal patterns of fish assemblages in non-tidal Mediterranean lagoons: A case study. *Estuarine, Coastal and Shelf Science* 83: 602–612.

Magalhães MC, Costa V, Menezes GM, Pinho MR, Santos RS, Monteiro LR 2007. Intra- ed inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from Azores. *Marine Pollution Bulletin* 54: 1654-1662.

Nybakken JW 1993. Marine biology: An ecological approach. HarperCollins College Publishers. New York, NY.

Quinn MR, Feng X, Folt CL, Chamberlain CP 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. *Science of the Total Environment* 317: 73-89.

Rainbow PS, Black WH 2005. Phytochemistry or psychology: Cadmium uptake and the effect of salinity and osmolality in three crabs of different ecologies. *Marine Ecology Progress Series* 286: 217-229.

Rauret G 1998. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta* 46: 449–455.

Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M 1999. Heavy metal distribution in different fish species from the Mauritania coast. *The Science of the Total Environment* 232: 169-175.

Rubio B, Gago L, Vilas F, Nombela M, Garcia-Gil S, Alejo I, Pazos O 1996. Interpretations of the historical trend of heavy metal pollution in sediment cores of the Pontevedra Estuary. *Thalassas* 12: 137–152.

Taylor A, Maher W 2003. The use of two marine gastropods, *Austrocochlea constricta* and *Bembicium auratum* as biomonitors of zinc, cadmium and copper exposure: effects of mass, withing and between site variability and net accumulation relative to environmental exposure. *Journal of Coastal Research* 19(3): 541-549.

Tittlemier SA, Fisk AT, Hobson KA, Norstrom RJ 2002. Examination of the bioaccumulation of halogenated dimethyl bipyrroles in an Arctic marine food web using stable nitrogen isotope analysis. *Environmental Pollution* 116: 85–93

Türkmen A, Türkmen M, Tepe Y, Akyurt Ý 2005. Heavy metals in three commercially valuable fish species from Ýskenderun Bay, Northern East Mediterranean Sea, Turkey. *Food Chemistry* 91(1): 167-172.

Zoumis T, Schmidt A, Grigorova L, Calmano W 2001. Contaminants in sediments: remobilization and demobilization. *Science of theTotal Environment* 266: 195–202.

Appendix A - Mean (±sd) trace metal levels (mg kg-1 d.w.) in fish from the three stations and four seasons in Acquatina.

		Cd			**Pb**			**THg**			**As**		
		South	**Central**	**North**	**South**	**Central**	**North**	**South**	**Central**	**North**	**South**	**Central**	**North**
Atherina boyeri	Summer	0.04±0.02	0.01±0.01	0.01±0.01	0.05±0.06	0.03±0.04	0.03±0.04	1.06±0.85	0.99±0.78	1.84±0.64	0.66±0.21	1.00±0.62	0.74±0.44
	Autumn	0.03±0.03	0.02±0.01	0.02±0.01	0.18±0.09	0.05±0.02	0.05±0.02	0.31±0.10	0.57±0.43	0.63±0.29	0.54±0.31	0.19±0.12	0.26±0.07
	Winter	0.06±0.05	0.06±0.02	0.07±0.03	0.19±0.14	0.07±0.02	0.13±0.05	0.30±0.19	0.89±0.39	0.48±0.20	0.65±0.37	0.34±0.14	0.63±0.46
	Spring	0.06±0.03	0.06±0.03	0.10±0.04	0.14±0.03	0.13±0.06	0.24±0.17	0.33±0.14	0.63±0.27	0.56±0.30	0.64±0.40	0.66±0.30	1.22±0.50
Chelon labrosus	Summer	0.03±0.01	0.05±0.01	0.05±0.04	0.15±0.04	0.10±0.03	0.11±0.03	0.10±0.04	0.23±0.06	0.19±0.03	0.52±0.10	0.47±0.21	0.64±0.29
	Autumn	0.03±0.01	0.02±0.01	0.04±0.02	0.08±0.02	0.07±0.04	0.08±0.04	0.09±0.02	0.09±0.029	0.15±0.06	0.43±0.13	0.23±0.20	0.34±0.13
	Winter	0.07±0.04	0.05±0.01	0.11±0.03	0.21±0.05	0.16±0.03	0.16±0.02	0.17±0.05	0.11±0.04	0.12±0.07	1.29±0.25	1.03±0.32	1.52±0.26
	Spring	0.03±0.01	0.08±0.04	0.03±0.01	0.06±0.01	0.25±0.04	0.07±0.04	0.12±0.03	0.18±0.08	0.08±0.05	0.44±0.09	0.91±0.37	0.30±0.15
Diplodus annularis	Summerr	0.05±0.02	0.04±0.01	-	0.08±0.02	0.08±0.04	-	0.39±0.07	0.14±0.08	-	0.58±0.08	0.33±0.12	-
	Autumn	0.04±0.01	0.05±0.011	-	0.15±0.03	0.07±0.03	-	0.44±0.07	0.13±0.06	-	0.44±0.070	0.35±0.03	-
	Winter	0.04±0.02	0.04±0.01	0.04±0.01	0.08±0.043	0.11±0.03	0.11±0.03	0.24±0.08	0.05±0.03	0.08±0.02	0.48±0.08	0.36±0.08	0.38±0.077
	Spring	-	-	-	-	-	-	-	-	-	-	-	-
Dentex dentex	Summer	0.06±0.01	0.04±0.02	-	0.07±0.01	0.07±0.05	-	0.30±0.24	0.30±0.13	-	0.71±0.26	0.52±0.15	-
	Autumn	0.03±0.01	0.03±0.01	0.03±0.01	0.07±0.01	0.06±0.02	0.07±0.05	0.47±0.09	0.14±0.07	0.03±0.01	0.22±0.00	0.30±0.18	0.42±0.06
	Winter	0.04±0.01	-	-	0.09±0.05	-	-	0.61±0.10	-	-	0.62±0.12	-	-
	Spring	-	-	-	-		-	-	-	-	-	-	-
Diplodus sargus	Summer	0.11±0.03	0.06±0.03	0.06±0.03	0.19±0.05	0.19±0.05	0.13±0.05	0.07±0.04	0.46±0.13	0.60±0.15	1.17±0.32	0.97±0.15	0.76±0.20
	Autumn	0.04±0.01	0.06±0.04	0.03±0.02	0.09±0.01	0.06±0.04	0.04±0.03	0.07±0.03	1.22±0.22	0.33±0.11	0.66±0.31	0.30±0.06	0.31±0.14
	Winter	0.07±0.02	-	-	0.14±0.04	-	-	0.05±0.02	-	-	0.72±0.10	-	-
	Spring	-	-	-	-	-	-	-	-	-	-	-	-
Diplodus vulgaris	Summer	0.03±0.02	0.02±0.02	-	0.06±0.02	0.08±0.03	-	0.19±0.07	0.18±0.05	-	0.35±0.25	0.51±0.05	-
	Autumn	0.03±0.02	-	-	0.03±0.02	-	-	0.13±0.05	-	-	0.33±0.20	-	-
	Winter	0.04±0.03	0.03±0.02	-	0.07±0.02	0.09±0.09	-	0.46±0.13	0.40±0.06	-	0.45±0.15	0.35±0.04	-
	Spring	0.05±0.02	0.10±0.04	-	0.07±0.02	0.35±0.07	-	0.21±0.07	0.58±0.12	-	1.13±0.16	1.16±0.32	-

Appendix A - Mean (±sd) trace metal levels (mg kg-1 d.w.) in fish from the three stations and four seasons in Acquatina.

		Cd			Pb			THg			As		
		South	**Central**	**North**	**South**	**Central**	**North**	**South**	**Central**	**North**	**South**	**Central**	**North**
Engraulis encrasicolus	Summer	0.04±0.01	0.05±0.02	-	0.01±0.01	0.07±0.03	-	0.55±0.20	0.47±0.11	-	0.41±0.14	0.31±0.07	-
	Autumn	-	-	0.03±0.01	-	-	0.07±0.02	-	-	0.23±0.03	-	-	0.25±0.05
	Winter	0.08±0.02	0.06±0.01	0.02±0.01	0.13±0.04	0.08±0.03	-	0.24±0.07	0.41±0.07	0.39±0.10	0.64±0.16	0.30±0.08	0.28±0.17
	Spring	0.06±0.02	-	-	0.03±0.01	-	-	0.27±0.07	-	-	1.10±0.22	-	-
Gobius paganellus	Summer	0.03±0.02	0.04±0.01	0.03±0.019	0.09±0.04	0.05±0.02	0.05±0.02	0.67±0.13	0.73±0.15	1.05±0.27	0.45±0.15	0.33±0.13	0.37±0.12
	Autumn	0.03±0.01	0.03±0.01	0.03±0.01	0.07±0.02	0.09±0.03	0.08±0.03	0.59±0.06	0.64±0.15	0.62±0.15	0.18±0.07	0.27±0.10	0.25±0.06
	Winter	0.05±0.01	0.03±0.01	0.03±0.01	0.06±0.05	0.13±0.01	0.07±0.02	1.13±0.17	0.45±0.11	0.77±0.23	0.32±0.18	0.41±0.14	0.45±0.11
	Spring	0.02±0.01	0.03±0.02	0.058±0.01	0.08±0.01	0.06±0.03	0.04±0.01	1.38±0.13	1.20±0.31	0.64±0.05	0.57±0.06	0.39±0.02	0.79±0.10
Liza ramada	Summer	0.02±0.02	0.06±0.03	0.04±0.02	0.01±0.01	0.04±0.01	0.02±0.01	0.27±0.08	0.57±0.09	0.41±0.15	0.56±0.13	1.29±.03	0.935±0.20
	Autumn	0.03±0.01	0.02±0.01	0.01±0.01	0.08±0.03	0.06±0.01	0.05±0.03	0.24±0.05	0.23±0.07	0.07±0.05	0.30±0.16	0.24±0.18	0.38±0.23
	Winter	0.03±0.02	0.02±0.01	0.04±0.01	0.06±0.01	0.05±0.01	0.05±0.02	0.20±0.07	0.23±0.05	0.07±0.04	0.38±0.08	0.22±0.09	0.35±0.09
	Spring	0.03±0.02	0.05±0.04	0.17±0.03	0.05±0.02	0.10±0.04	0.21±0.03	0.21±0.10	0.43±0.07	0.52±0.15	0.55±0.26	0.65±0.18	1.62±0.39
Mullus surmuletus	Summer	0.03±0.01	0.02±0.02	-	0.08±0.02	0.09±0.02	-	0.16±0.07	015±0.02	-	0.29±0.07	0.45±0.17	-
	Autumn	0.10±0.05	-	-	0.09±0.03	-	-	0.18±0.07	-	-	0.28±0.12	-	-
	Winter	-	-	-	-	-	-	-	-	-	-	-	-
	Spring	0.05±0.01	-	-	0.05±0.01		-	0.27±0.03	-	-	0.36±0.09	-	-
Sarpa salpa	Summer	0.12±0.03	0.04±0.01	0.03±0.01	0.21±0.05	0.10±0.03	0.09±0.04	0.36±0.09	0.44±0.08	0.13±0.08	0.80±0.13	0.39±0.08	0.32±0.07
	Autumn	0.03±0.01	0.01±0.01	0.03±0.01	0.08±0.03	0.09±0.02	0.07±0.03	0.19±0.09	0.21±0.07	0.07±0.02	0.34±0.14	0.39±0.09	0.45±0.20
	Winter	0.03±0.01	0.03±0.01	-	0.10±0.01	0.10±0.01	-	0.12±0.02	0.17±0.03	-	0.33±0.04	0.27±0.14	-
	Spring	-	0.10±0.02	0.12±0.03	-	0.15±0.02	0.30±0.06	-	0.35±0.06	0.47±0.12	-	0.83±0.14	0.73±0.19
Solea solea	Summer	0.05±0.02	0.04±0.01	-	0.08±0.01	0.06±0.01	-	0.20±0.07	0.02±0.01	-	0.39±0.07	0.33±0.07	-
	Autumn	0.03±0.01	0.02±0.01	-	0.06±0.01	0.05±0.01	-	0.14±0.03	0.01±0.01	-	0.05±0.01	0.19±0.10	-
	Winter	0.02±0.01	-	-	0.03±0.01	-	-	0.05±0.02	-	-	0.26±0.05	-	-
	Spring	-	0.04±0.01	-	-	0.05±0.01	-	-	0.04±0.01	-	-	0.13±0.03	-

As